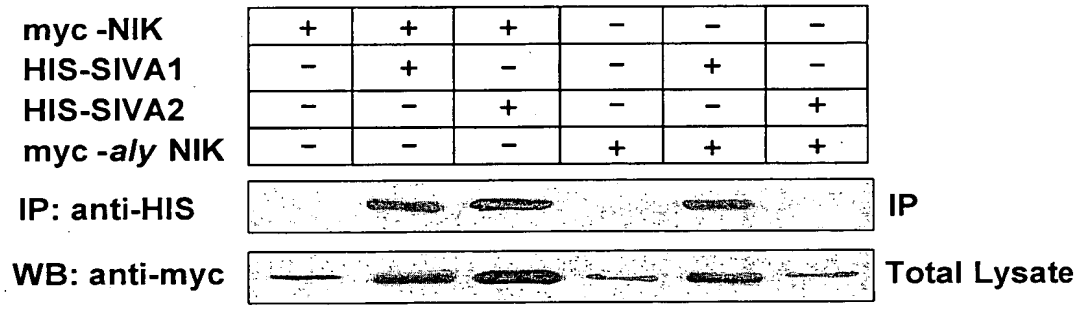
FIGURE 1

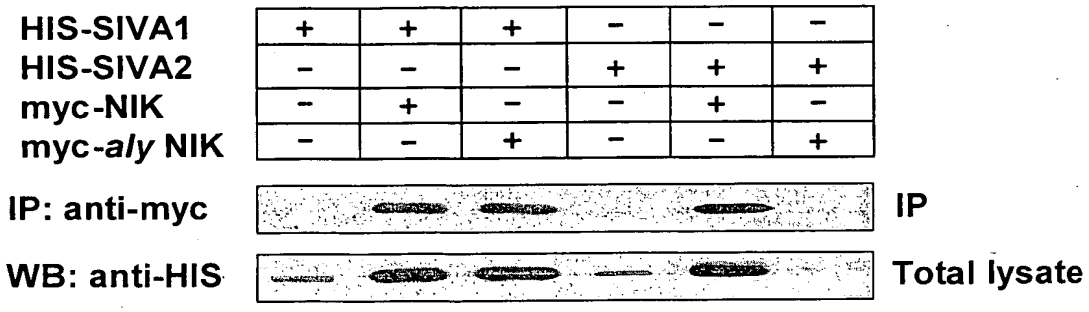
1A

Bait	Prey	Strength of interaction
NIK	SIVA-c	+
NIK	TRAF2	-
NIK-(624-947)	SIVA-c	++
NIK-(624-947)	TRAF2	+
SIVA-c	NIK-(624-947)	++

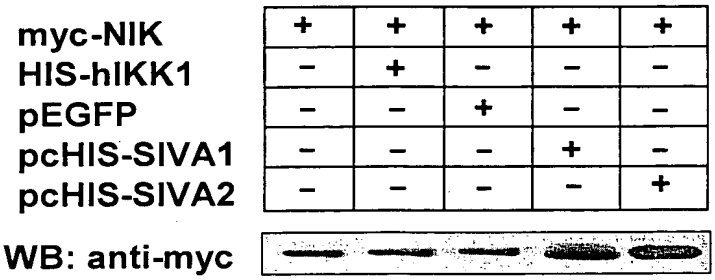
1B



1C



1D



1E

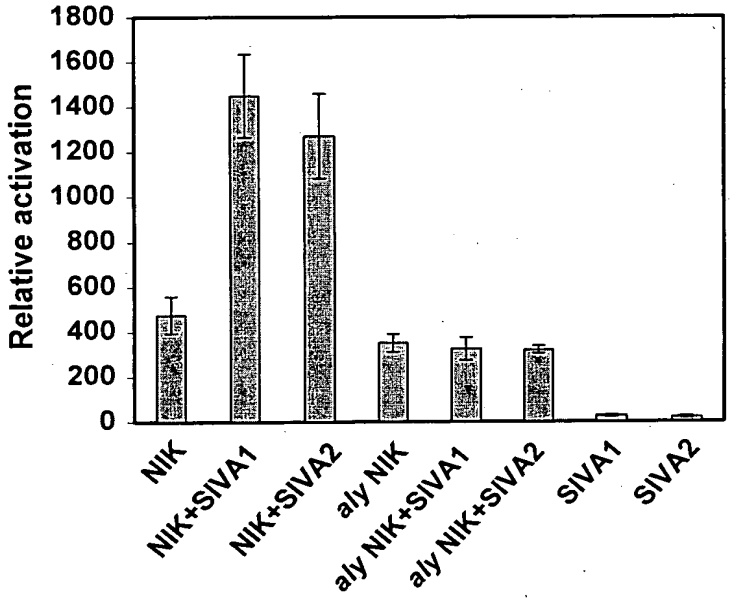
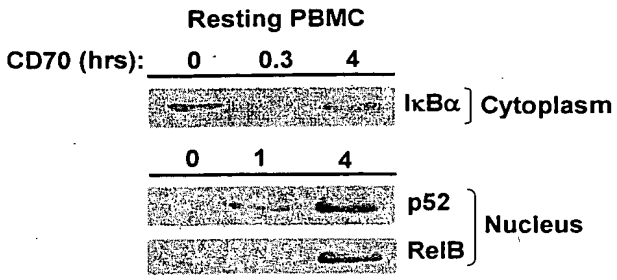
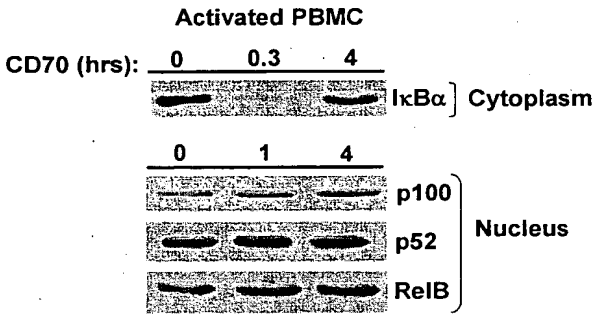


FIGURE 1

2A



2B



2C

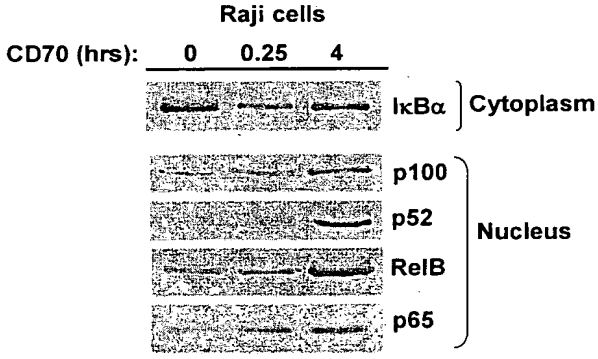
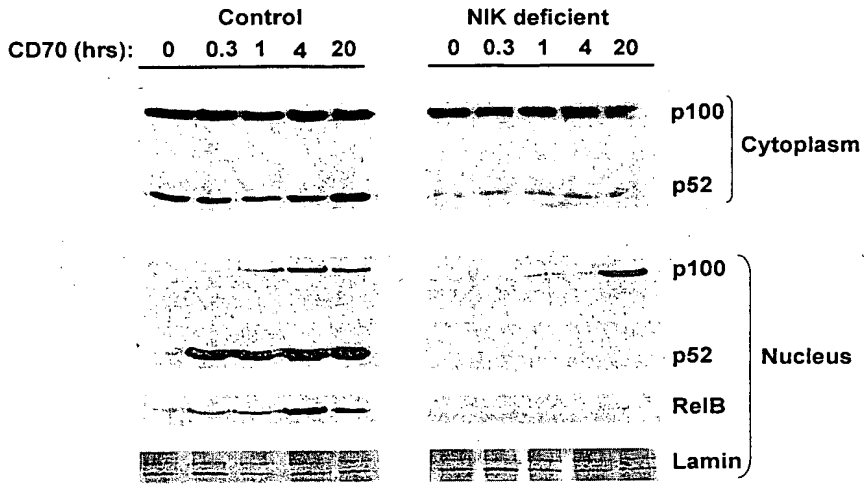
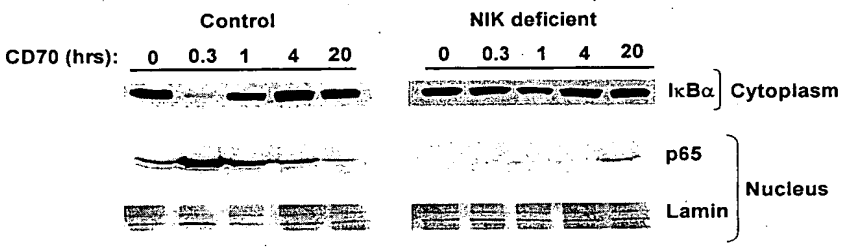


FIGURE 2

2D



2E



2F

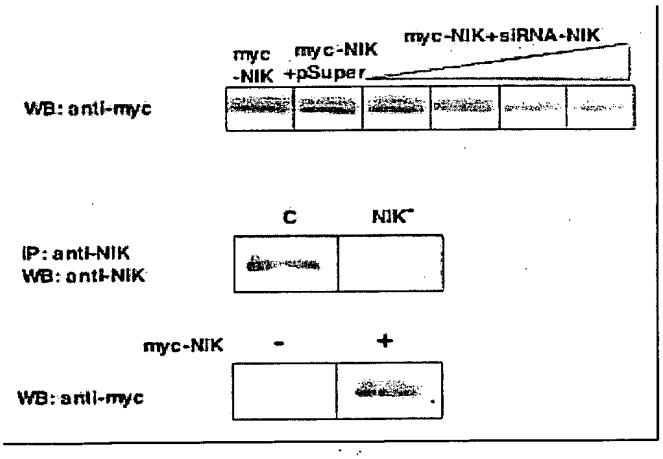
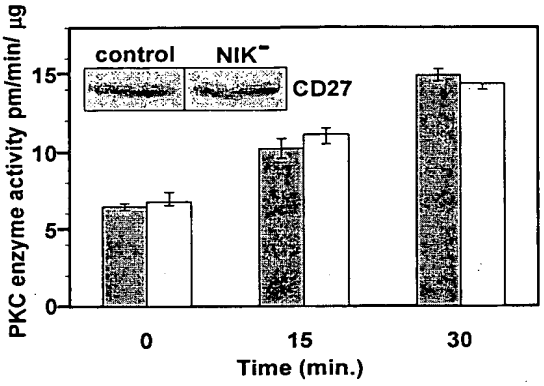


FIGURE 2

2G



2H

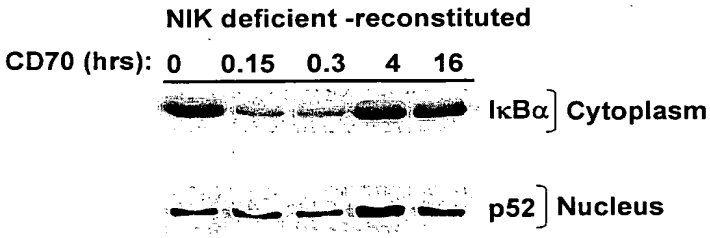
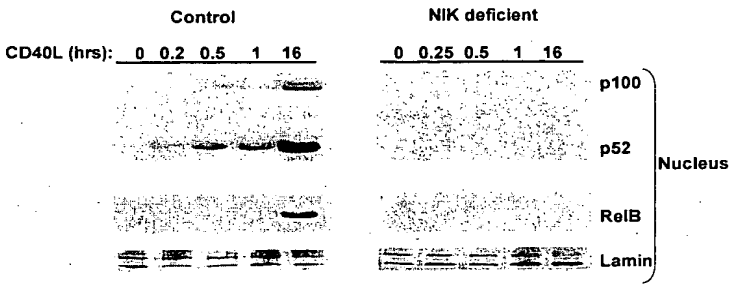
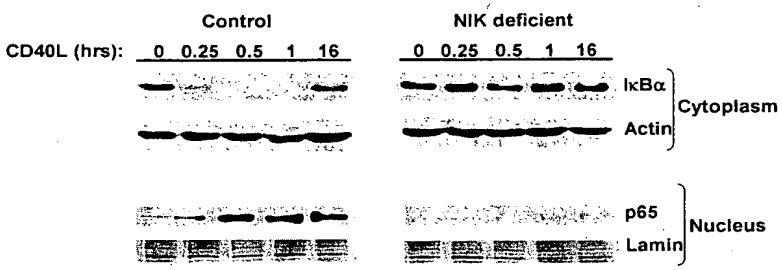


FIGURE 2

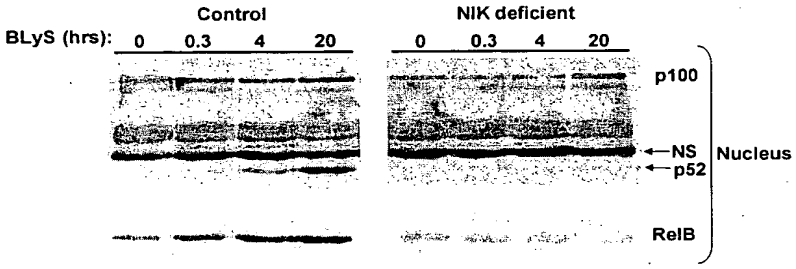
3A



3B



3C



3D

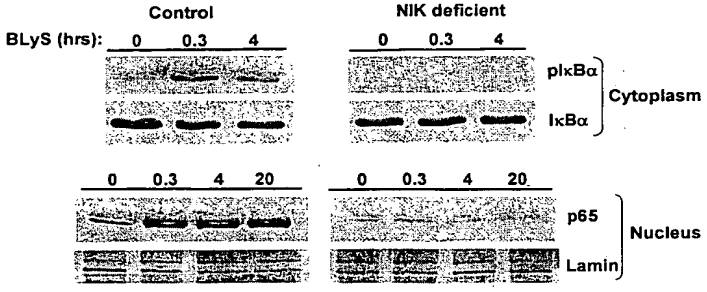
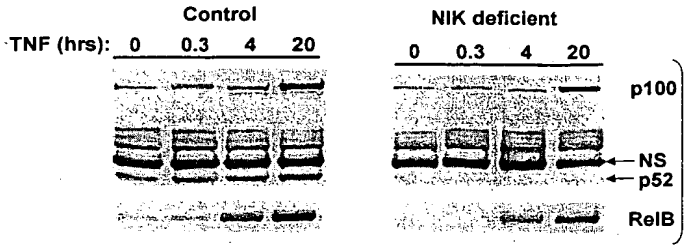
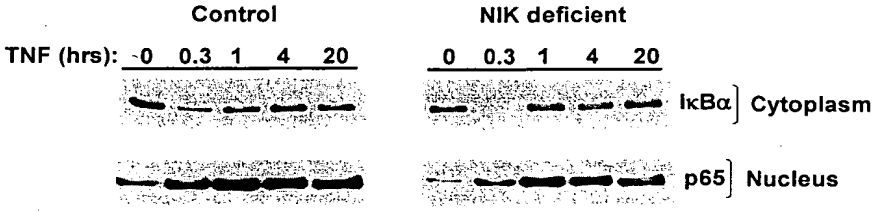


FIGURE-3

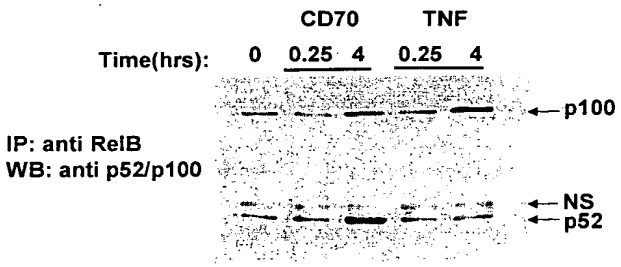
3E



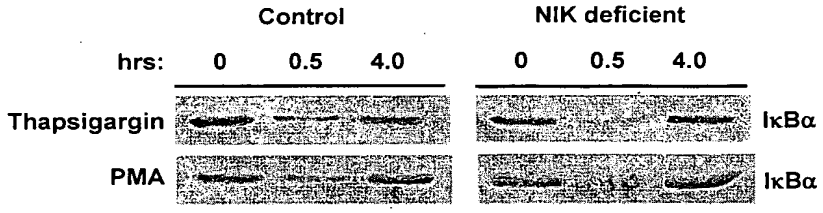
3F



3G



3H



3I

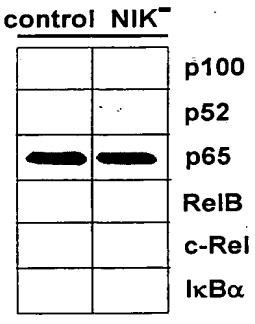
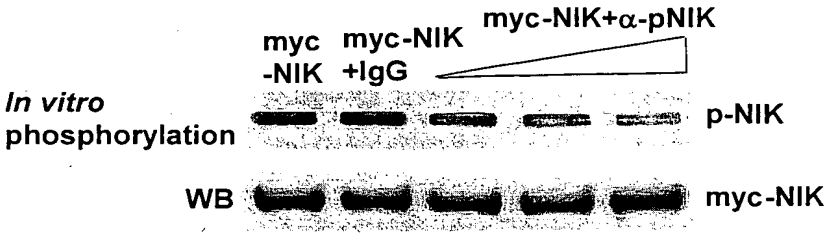


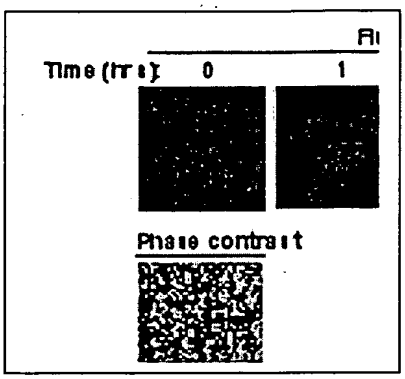
FIGURE-3

Figure 4

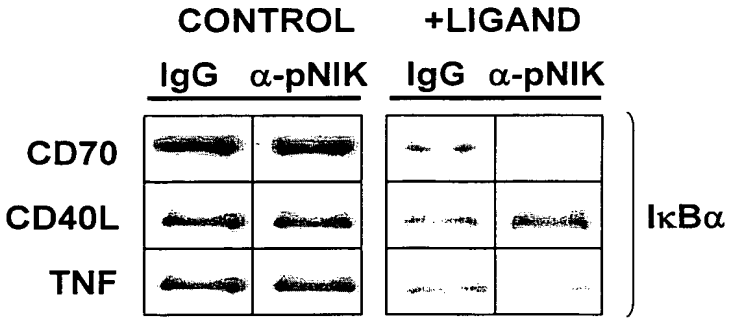
4A



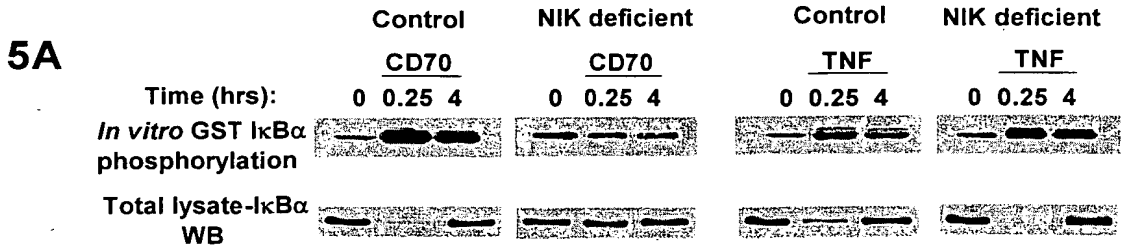
4B

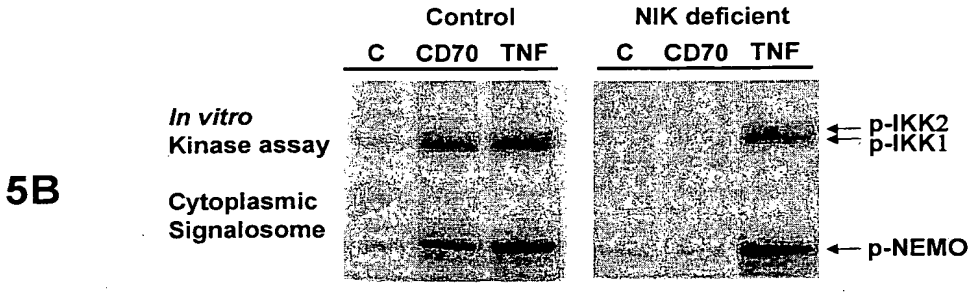


4C



4D


5A
Control
NIK deficient
Control
NIK deficient
CD70
CD70
TNF
TNF
Time (hrs):
0 0.25 4
0 0.25 4
0 0.25 4
0 0.25 4
In vitro GST IκBα phosphorylation
Total lysate-IκBα WB


5B
Control
NIK deficient
C CD70 TNF
C CD70 TNF
In vitro Kinase assay
Cytoplasmic Signalosome
p-IKK2
p-IKK1
p-NEMO

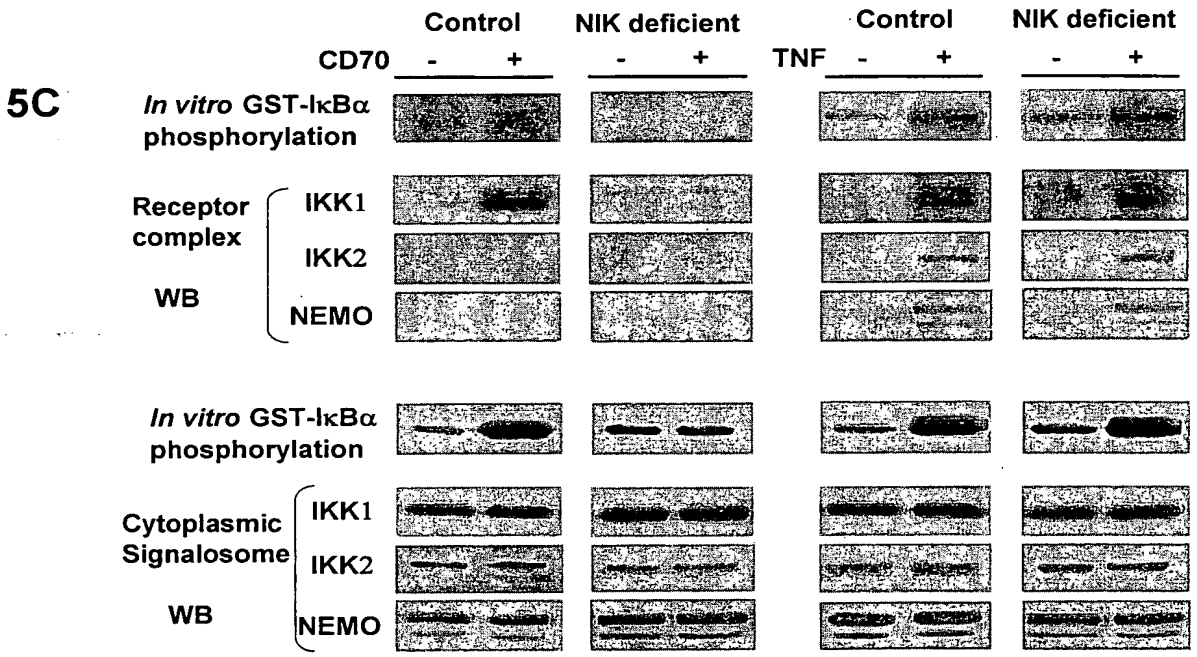
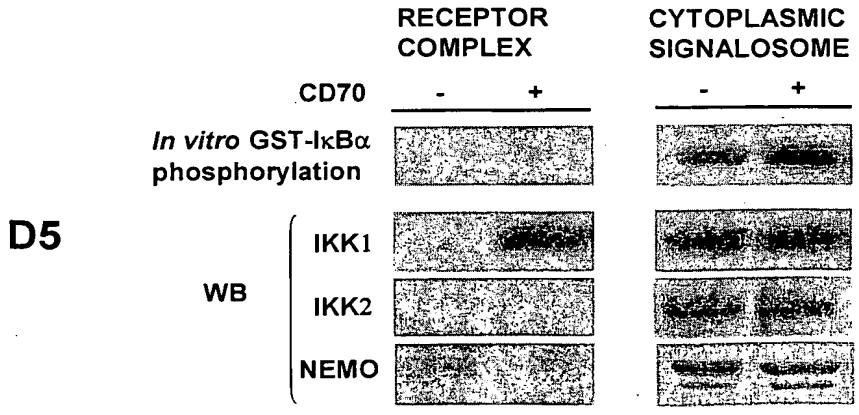

5C
Control
NIK deficient
Control
NIK deficient
CD70 - + - +
TNF - + - +
In vitro GST-IκBα phosphorylation
Receptor complex
IKK1
IKK2
NEMO
WB
In vitro GST-IκBα phosphorylation
Cytoplasmic Signalosome
IKK1
IKK2
NEMO
WB

FIGURE 5


D5
RECEPTOR COMPLEX
CYTOPLASMIC SIGNALOSOME
CD70 - + - +
In vitro GST-IκBα phosphorylation
IKK1
IKK2
NEMO
WB

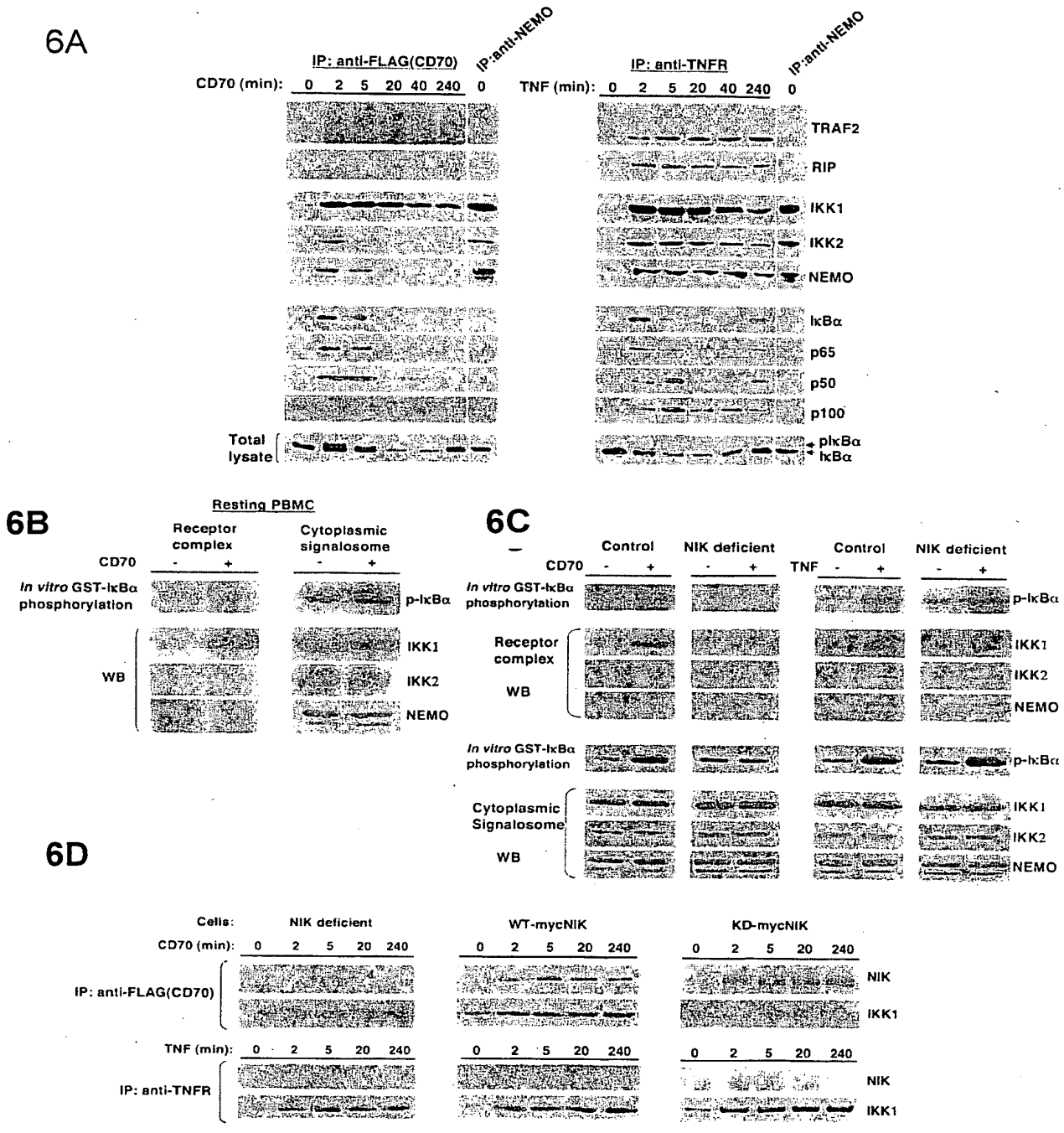

6A
IP: anti-FLAG(CD70)
IP:anti-NEMO
CD70 (min): 0 2 5 20 40 240 0
IP: anti-TNFR
IP:anti-NEMO
TNF (min): 0 2 5 20 40 240 0
TRAF2
RIP
IKK1
IKK2
NEMO
IκBα
p65
p50
p100
Total lysate
pIκBα
IκBα
6B
Resting PBMC
Receptor complex
Cytoplasmic signalosome
CD70
In vitro GST-IκBα phosphorylation
p-IκBα
WB
IKK1
IKK2
NEMO
6C
Control
NIK deficient
Control
NIK deficient
CD70
TNF
In vitro GST-IκBα phosphorylation
p-IκBα
Receptor complex
WB
IKK1
IKK2
NEMO
In vitro GST-IκBα phosphorylation
p-IκBα
Cytoplasmic Signalosome
WB
IKK1
IKK2
NEMO
6D
Cells:
NIK deficient
WT-mycNIK
KD-mycNIK
CD70 (min): 0 2 5 20 240
IP: anti-FLAG(CD70)
NIK
IKK1
TNF (min): 0 2 5 20 240
IP: anti-TNFR
NIK
IKK1

FIGURE 6

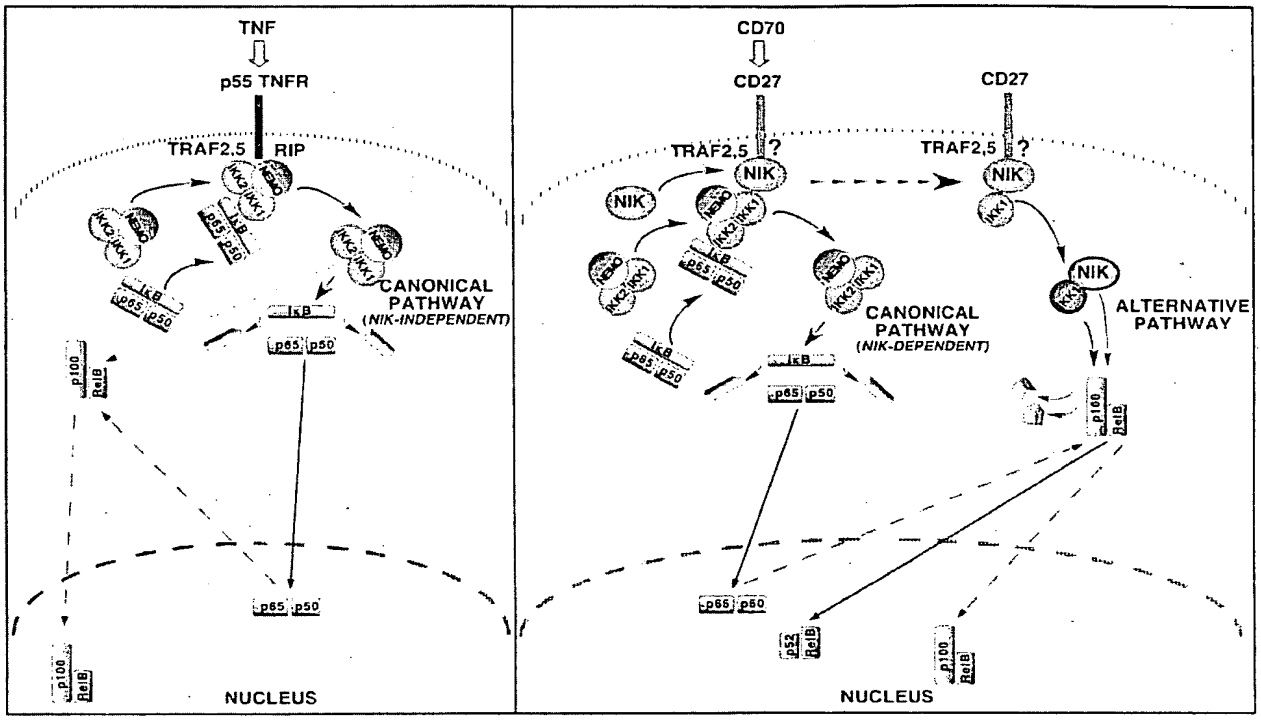

TNF
p55 TNFR
TRAF2,5
RIP
NEMO
IKK2
IKK1
IκB
p65
p50
CANONICAL
PATHWAY
(NIK-INDEPENDENT)
p100
RelB
NUCLEUS
CD70
CD27
CD27
TRAF2,5
?
NIK
CANONICAL
PATHWAY
(NIK-DEPENDENT)
ALTERNATIVE
PATHWAY
p52
RelB
NUCLEUS

FIGURE 7